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Figure 1A: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

OLIGONUCLEOTIDE	NUMBER	SIZE	SEQUENCE ¹	FEATURES
For 5' CTLA-4 amplification	3553 5023 5671	54	TTATTACTCgCggCCCAGCCggCCATggCCgCAATgCACgTggCC CagCCTgCT (SEQ ID NO:2)	forward primer + SfiI site
For 5' CTLA-4 amplification	5445	60	TTATTACTCgCggCCCAGCCggCCATggCCgCAATgCACgTggCC CagCCTgCTgTggTA (SEQ ID NO:3)	forward primer + SfiI site
For 5' CTLA-4 amplification	5443	45	TCTCACAgTgCACAggCAATgCACgTggCCCCAgCCTgCTgTggTA (SEQ ID NO:4)	forward primer + ApaLI site
For 5' CTLA-4 amplification	4851	39	TCTCACAgTgCACAggCAATgCACgTggCCCCAgCCTgCT (SEQ ID NO:5)	forward primer + ApaLI site
For 5' CTLA-4 amplification	5467	43	gCCCAGCCggCCgAATTcCAATgCACgTggCCCCAgCCTgCTg (SEQ ID NO:6)	forward primer + EcoRI site
For 5' CTLA-4 amplification		60	GCAGCTAATAGCACTCACTATAGGAACAGACCACCATGGA CGTGGCCCAGCCTGCTGTGG (SEQ ID NO:7)	ribosomal display
For 3' CTLA-4 amplification	4316 5022 5670	42	ATCTgCggCCgCTACATAAAATCTgggTACCgTTgCCgATgCC (SEQ ID NO:8)	reverse primer + NotI site
For 3' CTLA-4 amplification	4486	66	gCTgAATTCTgATCAGTgATggTgATggTgATgTgCggCCgCgTCag AATCTgggCACggTCTTgg (SEQ ID NO:9)	
For 3' CTLA-4 amplification		51	GCCCTTGGGCGGAGATGGTCTGTCTTCAGTGGCGAGGGC AGGTCTGTGTG (SEQ ID NO:10)	ribosomal display 1
For 3' CTLA-4 amplification		49	CGAGGGCAGGTCTGTGTGGGTCACGGTGCACGTGAACCTCT CCCCGGAG (SEQ ID NO:11)	ribosomal display 2

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Figure 1B: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

OLIGONUCLEOTIDE	NUMBER	SIZE	SEQUENCE	FEATURES
For 3' CTLA-4 amplification		51	CGTGAACCTCTCCCGGAGTTCCAGTCATCCTCGCAGATGC TGGCCTCACC (SEQ ID NO:12)	ribosomal display 3
For CDR1-somatostatin	4585	84	AgCTTTgTgTgAgTATgCagCTggCTgCAAgAATTCTTCTTgga AgACTTTTCACATCCTTgTgCCACTgAggTCCgggTgACA (SEQ ID NO:13)	forward primer + Som
For CDR3-somatostatin	4586	84	CTgggTACCgTTCCgATgCCACACAggATgTgAAAgTCTTCCAgAA gAAATTCTTgCagCCAgCCTCCACCTTgCAGATgTAGAg (SEQ ID NO:14)	reverse primer + Som
For CDR1-som-randomisation	4835	75	AgCTTTgTgTgAgTATgCagCTggCTgCAAgAATNNg/TNNg/T NNg/TNNg/TNNg/TNNg/TACATCCTTgTgCCACTgAggTC (SEQ ID NO:15)	
For CDR3-som-randomisation	4836	75	CTgggTACCgTTCCgATgCCACACAggATgTA/CNNA/CNNA/CNN A/CNNA/CNNA/CNNATTCTTgCagCCAgCCTCCACCTTgCA (SEQ ID NO:16)	
For CDR2 haemagglutinin tag	4766	21	gTAggTTgCCgCACAgACTTC (SEQ ID NO:17)	back primer for splice overlap
For CDR2 haemagglutinin tag	4775	66	gAAGTCTgTgCggCAACCTACCCgTATgACgTTCCggACTACgCC CTAgATgATTCCATCTgCACg (SEQ ID NO:18)	forward primer tag overlap
For CDR-1 anti-lysozyme	5232	78	gCCAgCTTTgTgTgAgTATgCCAgTggCTACACCATCgggCCgT ACTgCATgggCgTCCgggTgACAgTgCTTCgg (SEQ ID NO:19)	
For CDR-2 anti-lysozyme	5228	60	TgTgCggCagCCATCAACATgggCggTggCATCACCTTCCTAgAT gATTCCATCTgCACg (SEQ ID NO:20)	Forward

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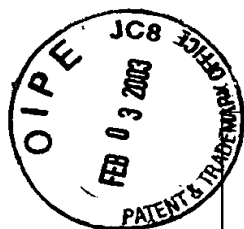


Figure 1C: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

OLIGONUCLEOTIDE	NUMBER	SIZE	SEQUENCE	FEATURES
For CDR-2 anti-lysozyme	5229	60	ATCTAggAAggTgATgCCACCgCCCATgTTgATggCTgCCgCACA gACTTCAgTCACCTg (SEQ ID NO:21)	Reverse
For CDR-3 anti-lysozyme	5230	69	CAGCCCGTggCCgCACTCgTAGTAggACgCgTAGATCgTcGAgTC CACCTTgCAGATgTAGAgTCCCgT (SEQ ID NO:22)	
For CDR-3 anti-lysozyme	5231	72	AATCTgggTACCgTTgCCgATgCCggAgTCATAgCCgTACCCTCC CgTggACAgCCCgTggCCgCACTCgTA (SEQ ID NO:23)	
For CDR-1 anti-melanoma	5341	78	gCCAgCTTTgTgTgTgATgCCAgTggATTCACTTCATgTTCC TACgCCATgTCCgTCCgggTgACAgTgCTTCgg (SEQ ID NO:24)	
For CDR-2 anti-melanoma	5338	51	gCCATCTCCggATCCggAggCTCgACCTACCTAgATgATTCCAT CTgCACg (SEQ ID NO:25)	Forward
For CDR-2 anti-melanoma	5339	54	gTAggTCgAgCCTCCggATCCggAgATggCTgCCgCACAgACTTC AgTCACCTg (SEQ ID NO:26)	Reverse
For CDR-3 anti-melanoma	5351	69	CACgTCCATgTAGTgTCTCCCTCCTCgCCgCgCgTCCCCAgC CCACCTTgCAGATgTAGTCCCgT (SEQ ID NO:27)	
For CDR-3 anti-melanoma	5340	51	AATCTgggTACCgTTgCCgATgCCCACgTCCCATgTAGTgTCTCC CTCCTC (SEQ ID NO:28)	
CDR1 Randomisation	4483	66	AgCTTTgTgTgATgTgCANNg/TNNG/TNNG/TNNG/TNNG/TN Ng/TNNG/TNNG/TgCCACTgAggTCCgggTgACA (SEQ ID NO:29)	forward primer / 8 residue random loop
CDR1 Randomisation	4484	68	CACgTggCCCAGCCTgCTgTggTACTggCCAgCgCCgAggCATCg CCAgCTTgTgTgTAGTATgC (SEQ ID NO:30)	extension primer
CDR1 Randomisation	4254	66	gTgTgTAGTACgCgTNCNNg/CNNg/CNNg/CNNg/CNNg/C TgCNNg/CgCTACTgAggTTCgTgTgACCgTC (SEQ ID NO:31)	forward primer with MluI, Afl II sites

Figure 1D: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

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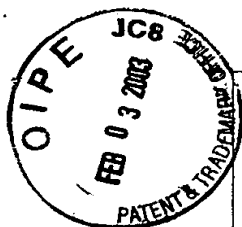


Figure 1E: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

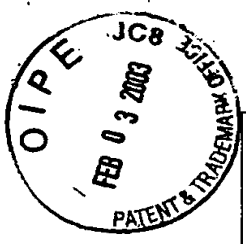
OLIGONUCLEOTIDE	NUMBER	SIZE	SEQUENCE	FEATURES
CDR3 Randomisation	5470	78	AATCTgggTACCCgTTgCCgATgCC(^Δ /cNN) ₁₀ CACCTTgCAGATgTAgATCCCCgT (SEQ ID NO:43)	
CDR3 Randomisation	5474	93	AATCTgggTACCCgTTgCCcAgATgCCCCAg(^Δ /cNN) ₁₃ CTCCACCTTgCAGATgTAgAgTCCCCgT (SEQ ID NO:44)	
CDR3 Randomisation	5471	81	AATCTgggTACCCgTTgCCgATgCC(^Δ /cNN) ₄ gCA(^Δ /cNN) ₆ CACCTTgCAGATgTAgAgTCCCCgT (SEQ ID NO:45)	
CDR3 Randomisation	5472	87	AATCTgggTACCCgTTgCCgATgCC(^Δ /cNN) ₃ gCA(^Δ /cNN) ₇ CACCTTgCAGATgTAgAgTCCCCgT (SEQ ID NO:46)	
CDR3 Randomisation	5475	99	AATCTgggTACCCgTTgCCgATgCC(^Δ /cNN) ₆ gCA(^Δ /cNN) ₁₀ CACCTTgCAGATgTAgAgTCCCCgT (SEQ ID NO:47)	
CDR3 Randomisation	5473	87	AATCTgggTACCCgTTgCCgATgCC(^Δ /cNN) ₄ gCA(^Δ /cNN) ₇ CACACCTTgCAGATgTAgAgTCCCCgT (SEQ ID NO:48)	
CTLA-4 codon-change	5591	70	ATgCACgTggCCCCAgCCTgTgTggTgCTggCCAgCgCCgTggCATgCCCCgCTTTgTgTgTgAAATATg (SEQ ID NO:49)	
CTLA-4 codon-change	5592	77	gCCAgCTTTgTgTgTgAAATATgCgTCTggCTATACCATCggCCCCgTACTgCATgggTgTgCgTgTgACCCgTgCTgCg (SEQ ID NO:50)	
CTLA-4 codon-change	5598	54	gTgCgTgTgACCCgTgCTgCgTCAggCggATAgCCAggTgACCCgAAgTTTgCgCg (SEQ ID NO:51)	
CTLA-4 codon-change	5600	75	CAGgTgACCCgAAgTTTgCgCggCgCgATCAACATgggCggTggCATCACCTTCCTggATgATTCCATCTgCACC (SEQ ID NO:52)	
CTLA-4 codon-change	5599	66	CAGACCCCTggATggTCAggTTCACCTggTTACCCgCTggAggTgCCgTgCAGATggAAATCATCCAg (SEQ ID NO:53)	
CTLA-4 codon-change	5597	57	CACTTTgCAGATgTACAgACCCgTATCCATggCACgCgACCCCTggATggTCAggTT (SEQ ID NO:54)	

Figure 1F: CTLA-4 VLD-SPECIFIC OLIGONUCLEOTIDES

CTLA-4 codon-change	5606	66	CAGGCCATgACCGCATTgTAATAAACgCATAgATggTgCTAT CCACTTTgCagATgTACAgACC (SEQ ID NO:55)	
CTLA-4 codon-change	5607	69	CTgggTACCgTTgCCgATgCCAGAAATCgTAgCCATAgCCACCggT ggACAaggCCATgACCGCATTgTA (SEQ ID NO:56)	

¹ All oligonucleotides are described 5' to 3'.
N represents combination of the four nucleotides.

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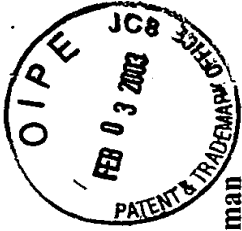


Figure 2: Polynucleotide sequence of complete cDNA encoding human CTLA-4 and polypeptide sequence of the VLD of human CTLA-4.

A. Polynucleotide Encoding Human CTLA-4 cDNA (SEQ ID NO:57)

```
ATGGCTTGCC TTGGATTTC ACGGACACAAG GCTCAGCTGA ACCTGGCTGC CAGGACCTGG CCCTGCACTC TCCTGTTTTT... 80
TCTTCTCTTC ATCCCTGTCT TCTGCAAAGC AATGCACGTG GCCCAGCCTG CTGTGGTACT GGCCAGCAGC CGAGGCATCG... 160
CCAGCTTTGT GTGTGAGTAT GCATCTCCAG GCAAAGCCAC TGAGGTCCGG GTGACAGTGC TTCCGGCAGG TGACAGCCAG... 240
GTGACTGAAG TCTGTGGGC AACCTACATG ACGGGGAATG AGTTGACCTT CCTAGATGAT TCCATCTGCA CCGGCACCTC... 320
CAGTGGAAAT CAAGTGAACC TCACTATCCA AGGACTGAGG GCCATGGACA CGGGACTCTA CATCTGCAAG GTGGAGCTCA... 400
TGTACCCACC GCCATACTAC CTGGGCATAG GCAACGGAAC CCAGATTAT GTAATTGATC CAGAACCCTG CCCAGATTCT... 480
GACTTCCTCC TCTGGATCCT TGCAGCAGTT AGTTCGGGGT TGTTTTTTA TAGCTTTCTC CTCACAGCTG TTTCTTTGAG... 560
CAAAATGCTA AAGAAAAGAA GCCCTCTTAC AACAGGGGTC TATGTGAAAA TGCCCCCAAC AGAGCCAGAA TGTGAAAAGC... 640
AATTTAGCC TTATTTTATT CCCATCAATT GA ... 672
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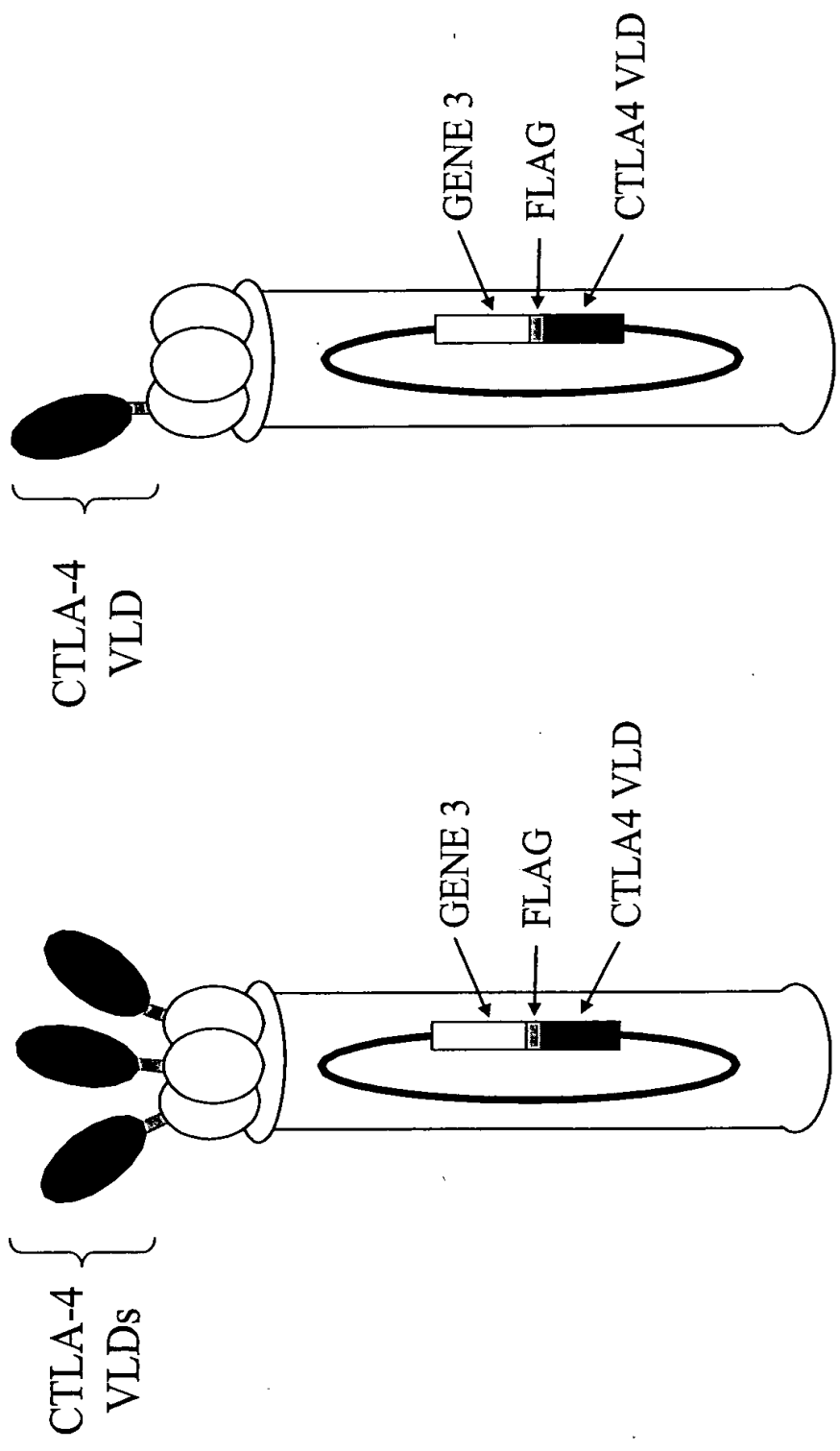
B. Polypeptide Sequence of Human CTLA-4 VLD (SEQ ID NO:58)

```
1 10 20 30 40 50 60 70 80
AMHVAQPAV LASSRGIAF VCEYASPGK TEVRVTVLRQ ADSQVTEVCA ATYMMGNELT FLDDSICTGT SSGNQVNLTI
90 100 110 115
QGLRAMDTGL YICKVELMYP PPYILGIGNG AQIYV
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Figure 3: Phage display of CTLA-4 VLD STMs

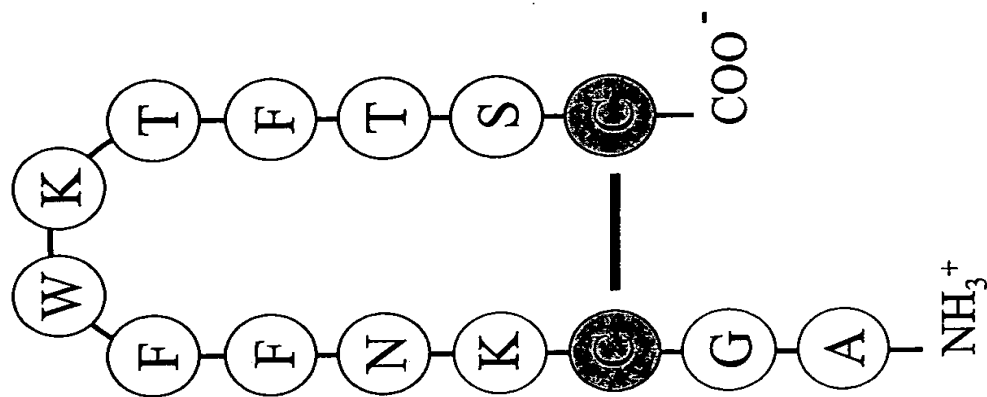


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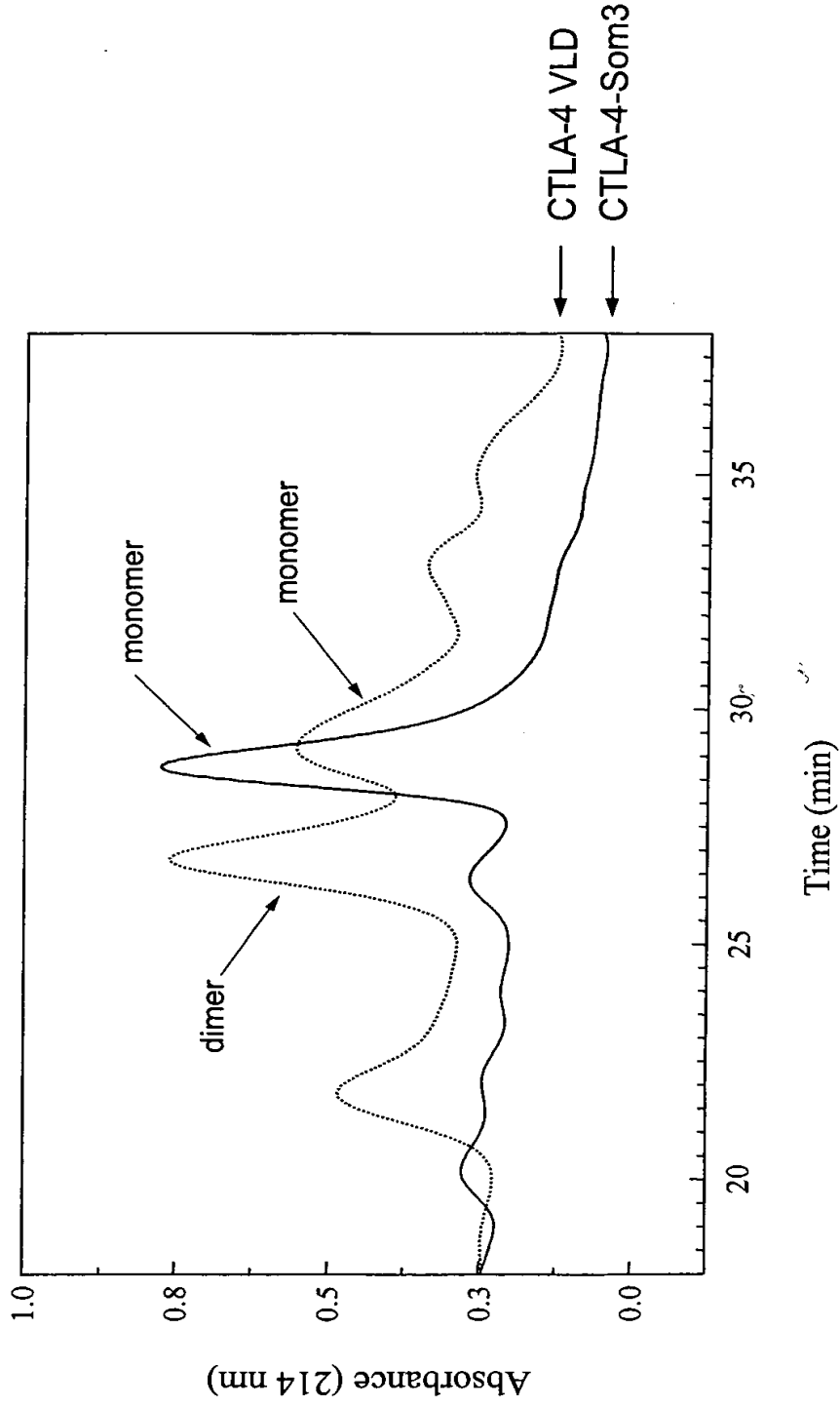
Figure 4: Schematic representation of the somatostatin peptide. (SEQ ID NO:139)

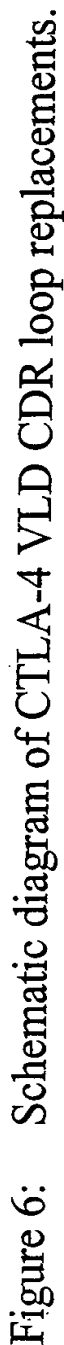


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Figure 5: Comparison of the HPLC profiles of affinity purified CTLA-4 VLD and CTLA-4-Som3 STM





A	CTLA-4 VLD	(S2)	SPGKATE	YMMGNELTF	LMYPPPPYYL
B	CTLA-4-Som1	(PP2)	AGCKNFFWKTFITSC	YMMGNELTF	LMYPPPPYYL
C	CTLA-4-Som1-tag	(PP5)	AGCKNFFWKTFITSC	YMMGNELTF	LMYPPPPYYL
D	CTLA-4-Som3	(PP8)	SPGKATE	YMMGNELTF	AGCKNFFWKTFITSC
E	CTLA-4-HA2	(XX4)	SPGKATE	YPYDVDPDYA	LMYPPPPYYL
F	CTLA-4-Som1-Som3	(VV3)	AGCKNFFWKTFITSC	YMMGNELTF	AGCKNFFWKTFITSC
G	CTLA-4-Som1-HA2-Som3	(ZZ3)	AGCKNFFWKTFITSC	YPYDVDPDYA	AGCKNFFWKTFITSC
H	CTLA-4-Anti-Lys	(2V8)	SGYTIGPYCMG	TYMMGNELTF	DSTIYASYECGHGLSTGGYGYDS
I	CTLA-4-Anti-Mel	(3E4)	SGTTFSSYAMS	AISGSGGSTY	GWGLRGEEGDYIMDV



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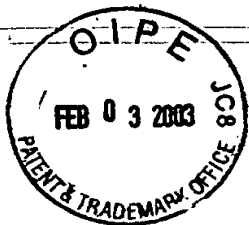
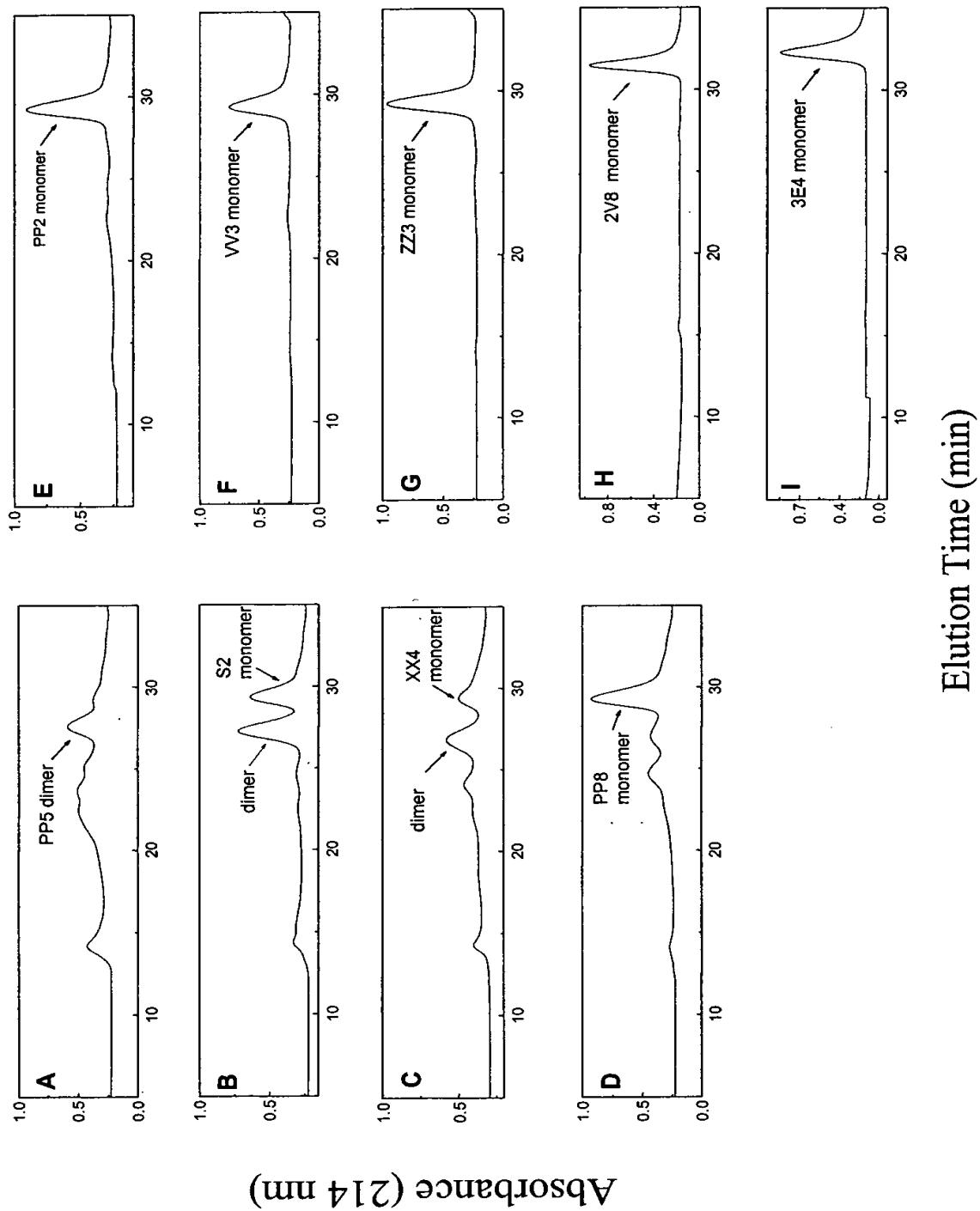


Figure 7: HPLC profiles of affinity purified recombinant CTLA-4 STMs



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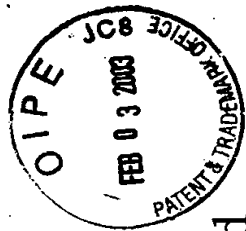
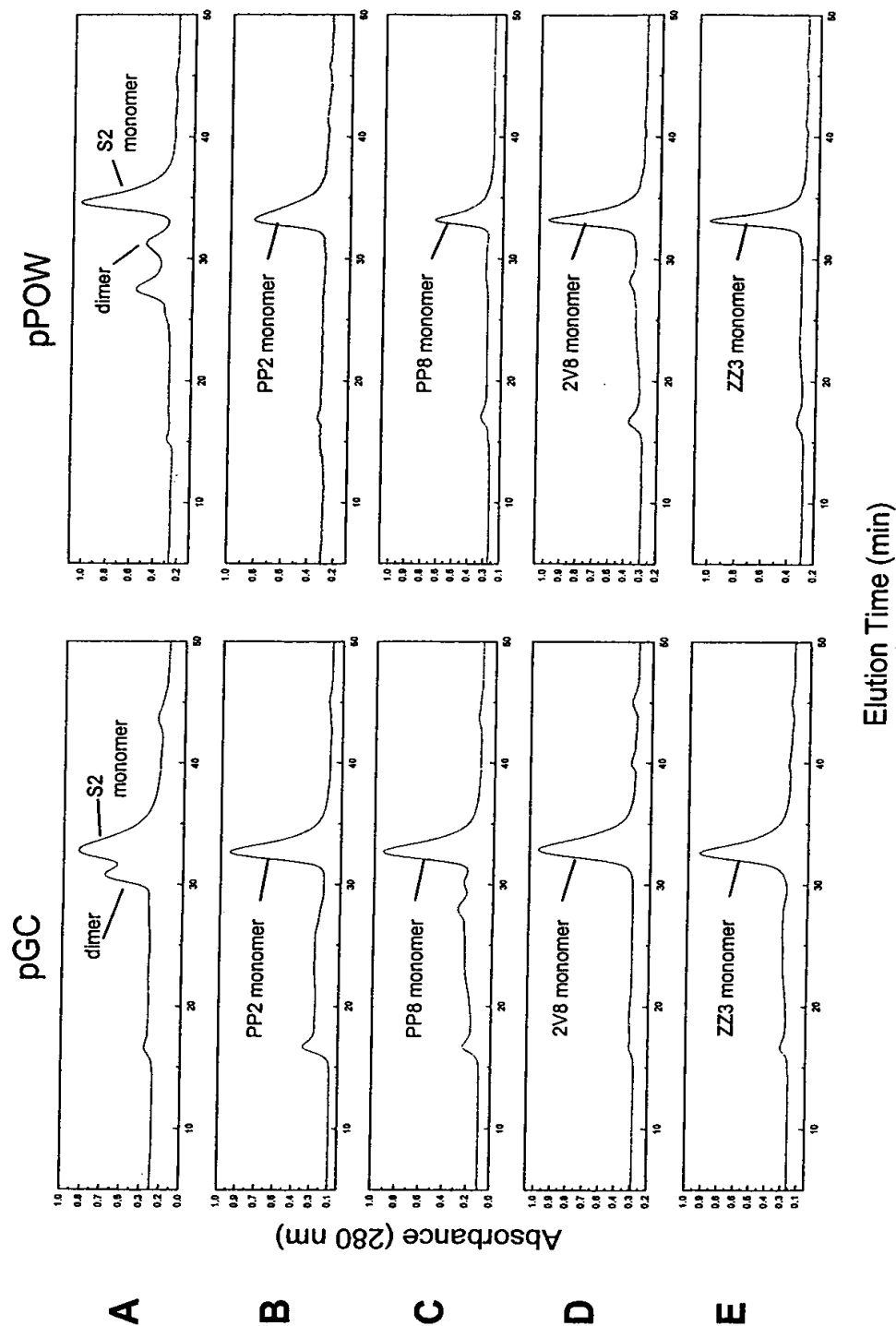
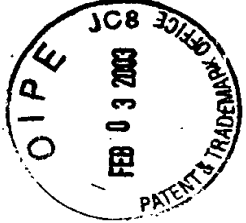


Figure 8:

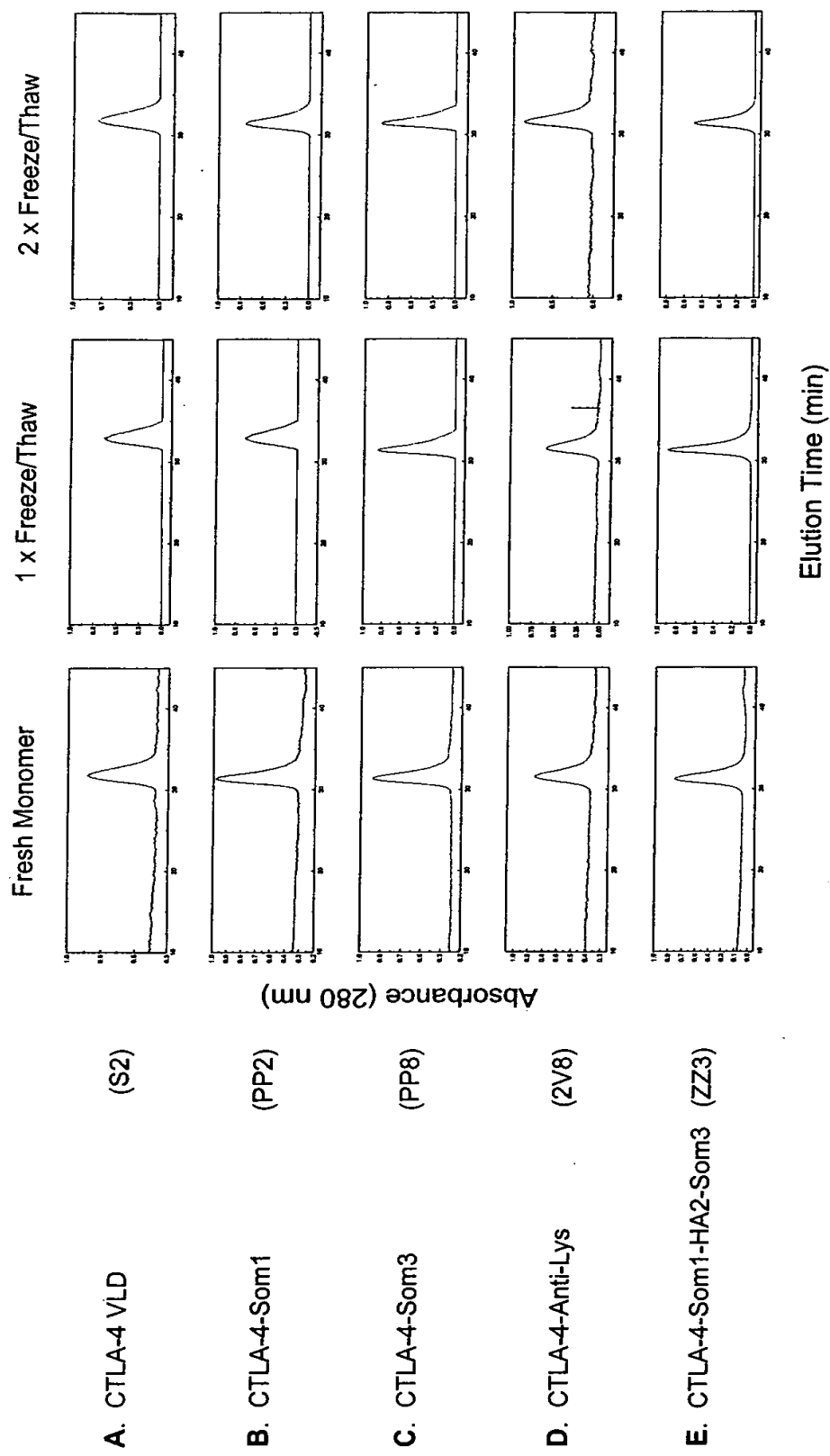
Comparison by size exclusion HPLC analysis of affinity purified CTLA-4 VLD STM constructs synthesised using bacterial expression vector pGC or pPOW.





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Figure 9: Size exclusion HPLC analysis of affinity purified CTLA-4 VLD STMs: Effect of Freeze/Thaw upon protein



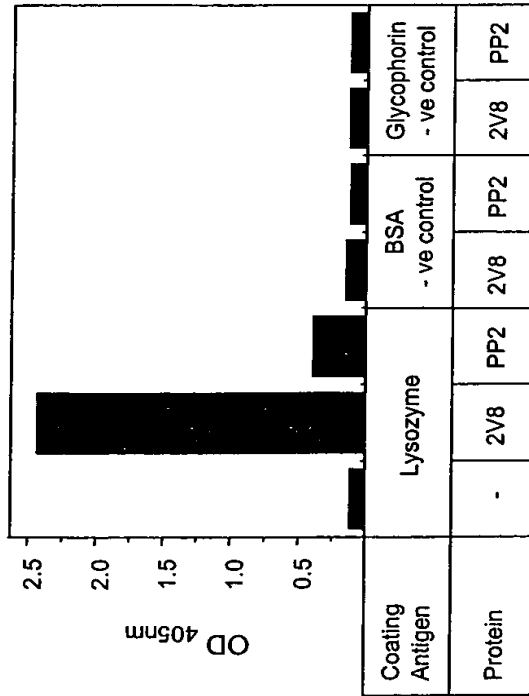
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Figure 10: Lysozyme binding characteristics of CTLA-4 anti-lysozyme construct 2V8

A

ELISA analysis of binding of 2V8 and PP2 constructs to lysozyme



B

BIAcore analysis of binding of lysozyme to 2V8 captured on immobilised anti-FLAG antibody

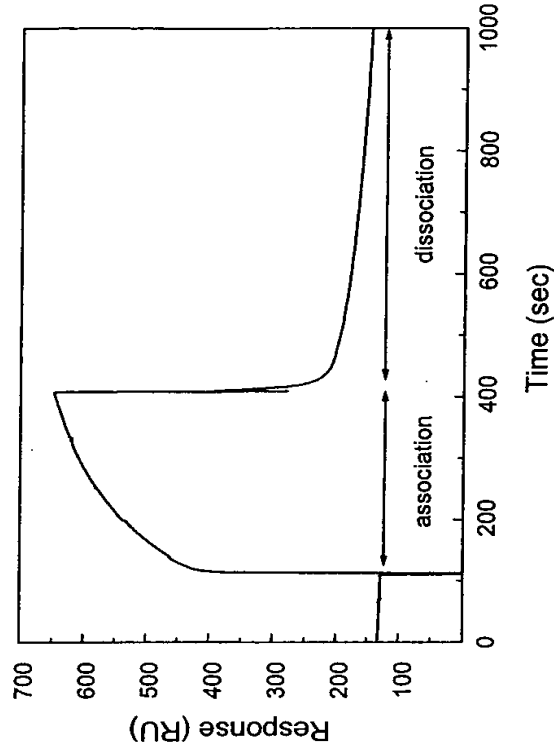
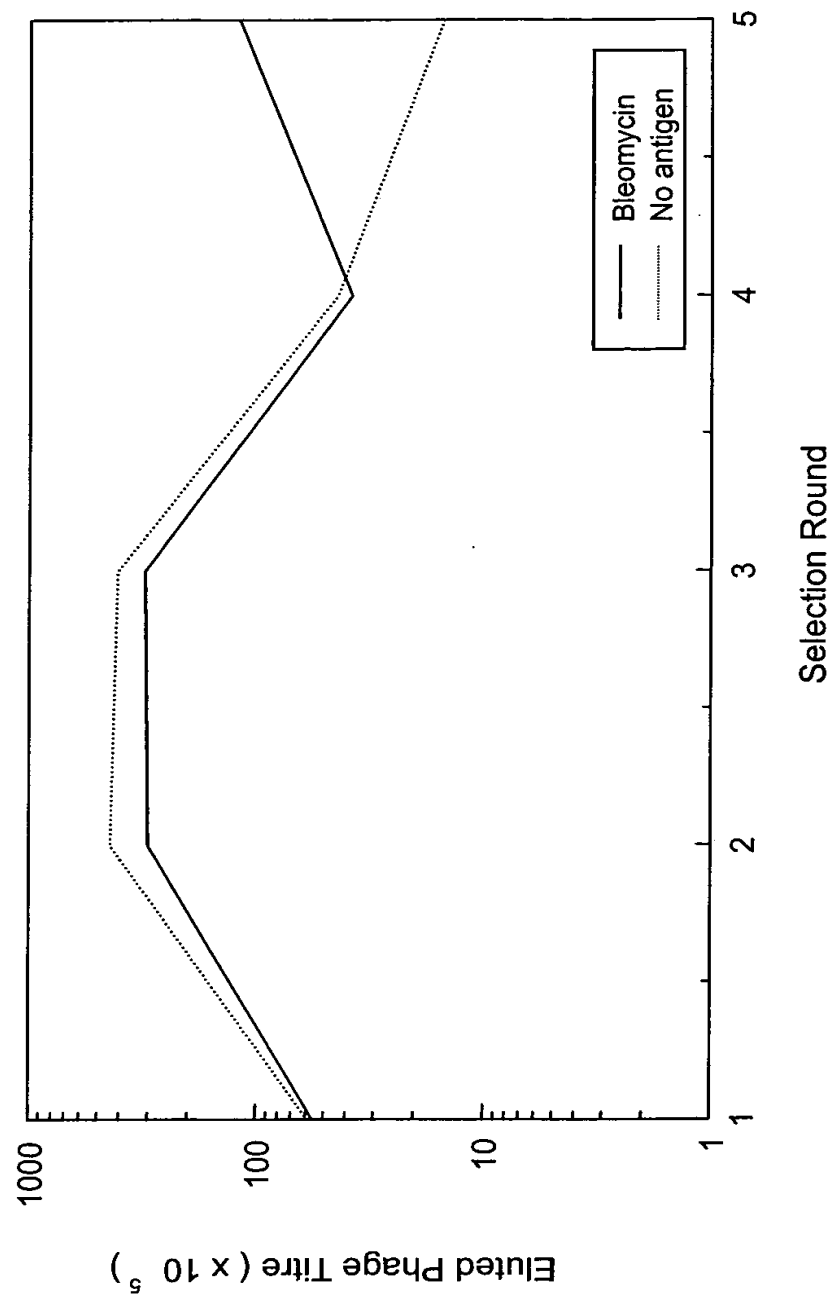


Figure 11: Screening of CTLA-4 VLD Phagemid Library on Immobilised Sh Bleomycin



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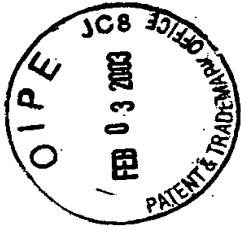


Figure 12: Screening of CTLA-4 VLD libraries in solution.

